Remarks

Claims 1-3, 10 and 18-21 are pending in the application. Claim 7 has been cancelled. Claims 4 to 6, 8 and 9 have been previously cancelled. Claims 11 to 17 have been withdrawn as a result of an earlier restriction requirement. Claims 18 to 21 have been added. No new matter has been added.

With respect to the rejection of claims 1 to 10 under 35 U.S.C. § 112, first paragraph, reconsideration by the Examiner is respectfully requested on the following grounds.

Scope of enablement:

1-propanol

First of all, the Applicant would like to remind the Examiner that the present invention relates to the increasing of the mutational rate of a DNA polymerase by the addition of an alcohol to the polymerase reaction mixture. All DNA polymerases have an intrinsic rate of mutation of about 0.0001 to 0.01%. The merit of the present invention is to provide a method for increasing this rate of mutation over the intrinsic values by addition of an alcohol. While the intrinsic rate of mutation of a DNA polymerase might represent a nuisance in some cases, other applications of PCR products, such as a large scale mutagenesis, are benefiting from such random mutations.

all DNA templates

It is submitted that no limitation has to be provided for DNA templates to be used with the current method, particularly since the current method is to be used in the context of a polymerization reaction, which is known in the art as being possible on any DNA template. The current method uses alcohol to increase the mutational rate of the DNA polymerase during the polymerization reaction. Thus it relates to the effect of the alcohol on the DNA polymerase and does not relate in any way to the DNA template. Therefore, it is submitted that the length of the DNA template to be used with the current method will be understood by the skilled man in the art as being equivalent to the length of any DNA template that can be used with a traditional polymerization reaction. Further, since the effect of alcohol on the DNA polymerase random mutational rate originates from the actual effect of the alcohol on the polymerase protein, as it is

known in the art that alcohol induces protein denaturation, the effect of the alcohol on the polymerase is unrelated to the DNA polymerase type.

1-propanol

Regarding the use of 1-propanol at concentration of 0.1%-15%, is it submitted that the amendment of claim 1 as limiting 1-propanol concentration to a maximum of 8% overcomes this rejection. Support for this amendment can be found in paragraph 0061 of the application.

Rejection of claim 7

Regarding the Examiner's comment that claim 7 allegedly requires that the generated mutant nucleic acid encode for a biologically active protein, it is submitted that the cancellation of claim 7 overcomes this rejection.

State of the art / unpredictability

Onto the section related to the state of the prior art, particularly regarding citations of Claveau *et al.* reported by the Examiner, it is respectfully requested that those citations are misinterpreted because cited outside of their context. For example, the Examiner cites page 791, right column, and paragraph 3 of Claveau *et al.*: "*...the deletion-to-mutation ratio induced by using Taq polymerase with 2.5% propanol was unsuitable for the desired goal*", the desired goal by Claveau et al. being "accelerated evolution" this does not means that use of 1-propanol on Taq polymerase fails to " induce random mutations into a nucleic acid" such as is claimed in claim 1. On this point, results presented in Claveau *et al.* agree that the use of propanol is suitable for that purpose, as mentioned in the sentence immediately preceding the one cited by the Examiner: "*For Taq polymerase in the presence of 2.5% propanol, ..., resulting in a raw mutation frequency of 9.8 x 10^{-4} mutation/bp/PCR which is higher than the intrinsic Taq polymerase error rate by a factor of 3 to 4*." (Emphasis added).

Regarding the citation of page 793, left column, paragraph 1, it is again submitted that since the purpose of the present invention is to increase the mutation rate of a DNA polymerase by addition of an alcohol, the invention works independently of the DNA template used. Therefore, while it might be possible that some very long DNA templates might not work in an optimal way with the present invention, those very long DNA templates will easily be recognized by the skilled man in the art for not working with traditional polymerization

techniques. In other words, because of the nature of the invention, the skilled man in the art will be able to predict that any DNA templates suitable for a traditional polymerization technique such as PCR will be suitable to be used with the current invention.

Regarding the third citation by the Examiner of the Claveau reference, it is submitted that the amendment of claim 1 to limit the 1-propanol concentration to a maximum of 8% renders this point moot.

Regarding the last citation by the Examiner of the Claveau reference, the citation from page 789, right column, it is submitted that the cancellation of claim 7 overcomes this rejection.

Level of skill, amount of guidance / experimentation

Regarding the comments of the Examiner on the level of one of ordinary skill, the amount of direction or guidance present, and the quantity of experimentation needed, it is submitted that, as reflected in the declaration of Marc Beauregard Ph.D. filed June 16, 2006, various independent laboratories have successfully used the methods claimed with various DNA templates. Since the mutation rate of the present methods is NOT related to the DNA template and the primers used in the polymerization reaction, it is submitted that the skilled man in the art would easily recognize that one only need to do one PCR experiment with a 10-wells plate followed by the sequencing of the clones obtained in order to identify the proper 1-propanol concentration for a desired rate of mutation, as reflected by the examples in the present application (see table 2 for an example). It is therefore submitted that, within the limits specific to polymerization techniques, sufficient guidance is provided to the skilled man in the art to successfully perform the methods claimed in its full scope as claimed in claim 1. Due consideration of the declaration of Dr. Marc Beauregard Ph.D. filed June 16, 2006, is earnestly solicited.

Claims rejection under §103

Regarding the rejection of claims 1-3, 7 and 10 as allegedly being unpatentable over Chevet *et al.* and Buchi, reconsideration by the Examiner is respectfully requested on the following grounds. First of all, the reference by Chevet *et al.* addresses an improved way to perform PCR with sub-optimal primers having extreme T_m. Chevet never even mentioned the

word mutation in his reference, concentrating his efforts on achieving an improved PCR assay. The mutational rate of the DNA polymerase is therefore viewed by Chevet as a decrease in the efficiency of the PCR assay, which is exactly the opposite of the goals of the present invention. While it is true that the Vent® polymerase used by Chevet possesses an inherent property of inducing random mutations, as evidenced by Keohavong *et al.*, it is respectfully reminded to the Examiner that the purpose of the present invention is to *increase* the intrinsic capacity of DNA polymerase to induce random mutations. Further, while Chevet uses ethanol in his polymerization reaction, it is submitted that the skilled person would not have thought of replacing ethanol by 1-propanol. On this point, in the office action dated December 19, 2005, pages 3 to 7, it was stated by the Examiner that the substitution of an alcohol for another cannot be considered obvious: "*It is not know how various alcohol molecules (such as propanol, ethanol, butanol, etc.) affect protein function and/or properties. In addition, the ultimate effect of alcohol on various polymerases (Vent, Taq, for examples) would be unpredictable.*" The Examiner can not have it both ways, either the substitution of alcohol is obvious or is not ! Therefore, it is submitted that the skilled man in the art, by reading the references by Chevet *et al.* in light of Buchi would in no way have been motivated to adding 0.1 to 8% 1-propanol to a polymerization reaction mixture in order to increase the mutational rate of a DNA polymerase above its intrinsic mutational rate as is currently claimed in claim 1.

Newly added claims

Claims 18 to 21 have been added based on the support found in paragraphs 0044 and 0046 of the disclosure.

Conclusion

It is therefore submitted that the claims are in condition for allowance. Reconsideration of the Examiner's rejections is respectfully requested. Allowance of claims 1-3, 10 and 18-21 at an early date is solicited.

The Applicant submits that no new matter has been added by way of the present amendment.

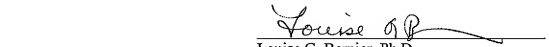
In the event that there are any questions concerning this amendment or the application in general, the Examiner is respectfully urged to telephone the undersigned so that prosecution of this application may be expedited.

No fees are believed to be required by the present response. However, should this be an error, authorization is hereby given to charge deposit account 19-5113 for any underpayment or to credit any overpayment.

Respectfully submitted,

AGROTERRA BIOTECH INC.

By:



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